Collaborative Study of Accuracy and Precision of Rapid Determination of Fat in Meat and Meat Products by Foss-Let Method

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Collaborators in 12 meat and food industry laboratories performed 4 fat determinations each on 7 samples of meat and meat products by the rapid (7–10 min) Foss-Let method and compared the results with those obtained by AOAC method 24.005(a) or 24.005(b). From the overall mean of results on all samples, determinations by the Foss-Let method averaged 0.11% fat higher than by the AOAC method. This difference was not significant by the *t*-test (P = 0.05), which indicated agreement between the compared methods in determining fat content. Precision of the Foss-Let method was equivalent to and generally slightly better than that of the AOAC method. Standard deviations with the Foss-Let method were 0.2% fat for between-duplicates and for within-laboratory repeatability; 0.4% fat for between-laboratories, including variation due to laboratory-sample interaction; and 0.5% fat for reproducibility between analysts in different laboratories. The Foss-Let method has been adopted as official first action.

We reported consideration of the Foss-Let method as an alternative to AOAC method 24.005(a) or 24.005(b) (1) in a previous communication (2). The critical evaluation in our laboratory demonstrated the reliability of the method and accuracy equivalent to the AOAC method. Its performance in providing rapid (7–10 min) and convenient fat determination indicated its potential usefulness to meat analysts.

The present collaborative study determined accuracy and precision of the Foss-Let method within and among laboratories following a prescribed procedure. The study yielded information on 5 components of precision: between duplicate determinations, repeatability between different days, laboratory-sample interaction, between laboratories, and reproducibility. We then compared the characteristics with those established and reported earlier (3) for the AOAC method on the same set of samples.

Collaborative Study

Collaborators participating in this study were analysts in meat and/or food industry laboratories who were experienced with the Foss-Let method in their normal operations. Independent determinations were performed by an analyst at each of 12 locations, using his equipment and supplies. Samples representative of products encountered in meat packing and processing and regulatory work were prepared by method 24.001 (1), packed in plastic bags, and distributed frozen to the collaborators. The samples were portions of the same ones distributed to the collaborators for analysis by ether extraction (3) to evaluate the method on both fresh meat and emulsified meat products: 3 beef (about 10, 20, and 25% fat), 2 pork (about 3.5 and 48% fat), 1 frankfurter (about 27% fat), and 1 bologna (about 22% fat). Fresh meat samples were prepared from lean and fatty tissue of commercial beef and pork purchased from local packers. Frankfurter and bologna samples were prepared from quantities of commercial lots purchased from local processors. The collaborators were requested to store the samples frozen until analyses were to be performed, then thaw the sample required, transfer it to a vessel and thoroughly remix it, return the unused portion of the sample to its original plastic bag or to a similar one, and refrigerate but not refreeze it until ready for the second

analysis. Collaborators were requested to perform duplicate analyses on a sample, or samples, on one day and repeat determinations in duplicate within less than a week. They were requested to use the filter paper supplied to them for convenience and uniformity, Whatman No. 50 and No. 1PS, 7 cm D, in the filtration step of the procedure. All determinations were to be recorded to the second decimal place to prevent loss of significance in the first decimal place, which is the limit of accuracy and precision of fat determination when expressed as per cent of composition.

The principle of the Foss-Let method, apparatus for performing determinations, and analytical procedure applied by the collaborators were described in the background report (2) of our own evaluation of the method.

Results and Discussion

The collaborative results were statistically treated following the procedures of Youden (4), Youden and Steiner (5), and the American Society for Testing and Materials (6, 7). Outlier tests statistically indicated suspect data or laboratories to be excluded from calculation of components of precision. Precisions of duplicate determinations calculated relative to each sample were pooled to obtain overall results by weighting the sample variances for degrees of freedom after testing for homogeneity by Bartlett's chi-square test (8). Two different analyses of variances, each designed to yield discrete information, determined other precision characteristics of the Foss-Let method. One analysis of variance calculated between-days repeatability, compound between-laboratory variation, and reproducibility relative to each collaborative sample. These 3 precisions, after being tested for homogeneity by Bartlett's chi-square test and the F_{max}-test (9), were pooled for all samples to obtain overall expressions of the precisions. The overall expressions were then compared with similar results obtained, already pooled, from a second analysis of variance involving all 7 samples in one block of data. This second analysis also resolved the laboratory-sample interaction component and the simple between-laboratory variation as separate components of the compound between-laboratory variation calculated with the former analysis of variance. Accuracy of the Foss-Let method was assessed

by comparing sample means with corresponding AOAC ether extraction determinations (3).

Outlier Tests

Collaborative determinations (Table 1) were examined by ranking the data, constructing 2-sample plots, and testing the differences between duplicate determinations, between days, and among laboratory averages. From results of these tests, 10 duplicate determinations from Collaborator 5 were excluded in calculating the between-duplicates precision and all data from the collaborator were excluded from analyses of variance. The data were treated both with and without an outlier in data from Collaborator 9, as will be indicated.

Standard Deviation Between Duplicate Determinations

Sample means, variances, standard deviations, and pooled results were calculated from the duplicate differences (Table 2). Homogeneity of variance was tested, and 6 of the sample variances were homogeneous after the lowest variance, corresponding to Sample P-1, was excluded from the calculation. This test indicated that the precision of duplicate determinations, in terms of standard deviation, was 0.1% fat at the 3.5% fat level and 0.2% at higher levels up to about 48% fat.

Precision of duplicate determinations was relatively constant for different fat contents when expressed in terms of standard deviation (absolute). In terms of coefficient of variation (relative), where coefficient of variation = 100 × standard deviation/mean % fat, the variation ranged from 2.65% for the lowest fat sample to 0.33% for the highest.

The maximum range for duplicate determinations acceptable at a 95% confidence level (Table 2) was calculated to be ±0.3% fat at the 3.5% fat level and ±0.5% at higher levels up to about 48% fat.

Analysis of Variance from Single Sample Data Sets

Collaborative duplicate determinations were averaged so that day averages were used as replicates to calculate 3 sums of squares and 2 mean squares for each sample. Sample variances and pooled results for all samples and homogeneous groups were calculated from th

Table 1. Collaborative study of fat (%) in meat and meat products by Foss-Let method

Coll.	Beef-1 Day 1	Beef-1 Day 2	Beef-2 Day 1	Beef-2 Day 2	Beef-3 Day 1	Beef-3 Day 2	Pork-1 Day 1	Pork-1 Day 2	Pork-2 Day 1	Pork-2 Day 2	Frankfurter Day 1	Frankfurter Day 2	Bologna Day 1	Bologna Day 2
1 (AR)	10.72	11.28	20.01	20.00	25.72	25.78	3.75	3.68	48.64	48.31	27.75	27.43	22.36	22.16
	10.67	11.17	19.87	20.46	25.44	25.64	3.83	3.52	48.65	48.42	27.44	27.37	22.54	22.25
2	10.78	10.90	20.31	20.13	25.02	24.09	3.38	3.26	48.20	48.20	26.60	26.76	22.56	21.86
	10.92	10.94	20.40	20.32	24.87	24.14	3.34	3.28	48.20	48.22	26.56	26.75	22.11	22.32
3	10.75	10.95	20.50	20.40	25.50	25.55	3.40	3.55	49.10	49.10	26.95	27.25	22.30	22.90
	10.75	10.85	20.65	20.40	25.50	25.40	3.45	3.55	49.25	48.95	27.25	27.50	22.35	22.75
4	11.20	10.90	20.85	20.55	26.05	25.70	3.40	3.30	50.00	50.30	28.15	28.05	23.70	23.70
	11.30	10.90	21.10	20.75	26.00	25.75	3.40	3.35	50.05	50.40	28.05	28.05	23.65	23.70
5	6.20	14.05	13.65	15.90	23.80	20.90	6.30	4.95	22.50	30.90	31.25	24.65	26.25	25.30
	9.00	12.95	17.65	19.65	27.10	10.95	5.15	2.70	22.30	12.60	31.05	28.50	26.15	24.75
6	10.83	10.80	20.50	20.80	25.93	26.05	3.43	3.35	49.20	49.26	27.20	27.65	22.75	22.95
	10.50	10.90	20.80	21.18	26.18	26.20	3.43	3.33	49.20	48.96	27.08	27.45	23.10	23.20
7	11.10	11.05	19.90	19.70	25.40	25.35	3.55	3.75	48.35	48.75	27.10	27.05	22.55	22.30
	10.80	10.85	20.30	19.90	24.90	25.20	3.40	3.40	48.65	48.75	26.95	26.95	22.35	22.45
8	10.07	10.05	20.50	20.55	25.45	25.40	3.40	3.40	48.35	48.30	26.65	26.60	22.02	21.95
	10.07	10.05	20.55	20.55	25.45	25.40	3.40	3.40	48.35	48.25	27.00	26.65	22.00	22.02
9	10.85	10.75	20.00	20.30	25.05	25.55	3.35	3.65	48.50	49.35	26.45	26.55	22.35	22.65
	10.70	10.30	19.65	20.00	25.25	25.95	3.70	3.80	48.70	50.05	27.10	27.33	22.65	22.85
10	10.65	10.10	20.55	20.45	25.35	25.30	3.75	3.65	48.50	48.50	27.40	27.25	22.60	22.15
	10.30	10.10	20.50	20.50	25.40	25.25	3.60	3.70	48.70	48.80	27.25	27.10	22.70	22.20
11	11.50	10.90	20.45	20.50	25.70	25.40	3.45	3.40	50.05	49.35	27.95	27.95	23.05	22.60
	11.55	11.05	20.65	20.65	25.85	25.35	3.45	3.45	49.95	49.30	27.55	28.10	23.20	22.95
12	10.80	10.80	20.95	21.50	25.85	25.75	3.55	3.45	48.80	49.25	26.50	26.55	22.55	22.50
	10.75	10.80	20.85	21.15	25.85	25.95	3.40	3.50	49.25	49.30	26.50	26.35	22.55	22.55

Table 2. Statistics of precision of duplicate determinations on samples individually and pooled

Sample	Mean, % fat	No. of dupl. pairs	Variance	Std dev., % fat	Max. range for dupls.[a]
B-1	10.77	22	0.0154	0.12	0.35
B-2	20.47	22	0.0284	0.17	0.50
B-3	25.50	22	0.0176	0.13	0.38
P-1	3.49	22	0.0086	0.09	0.26
P-2	47.82	23	0.0254	0.16	0.47
Fr	27.36	23	0.0394	0.20	0.59
Bol	22.87	24	0.0278	0.17	0.50
All samples	22.61	158	0.0234	0.15	0.42
All samples except P-1	25.80	136	0.0258	0.16	0.45

[a] 95% confidence level, ±% fat, calculated by multiplying standard deviation by a factor (6, p. 520) which consists of ($\sqrt{2}$) ($t_{0.5}$) for the corresponding degrees of freedom.

mean squares (Table 3). Both the within-laboratory and summed (reproducibility) sample variances were homogeneous after excluding the variance of the low fat sample, P-1. Between-laboratory sample variances were homogeneous in 2 subgroups, divided according to fat content: a lower variance for the 2 samples, B-1 and P-1, low in fat content, and a higher variance for the 5 samples higher in fat content.

Components of precision summarize the statistics of this analysis of variance expressed in terms of standard deviation and relative deviation (Table 4). Collaborative means of fat content shown in the first column of data differ in some cases from those in Table 2 as a result of having excluded different outlier data from the treatments. As in the case of precision of duplicate determinations discussed above, repeatability and reproducibility values formed a relatively narrower range when expressed as standard deviation rather than as relative deviation. From the pooled results of standard deviation,

| | Variance | | | |
| | s_a^2 (within-lab.) | s_b^2 (between-lab.) | Sum $s_a^2 + s_b^2$ | F-ratio, s_b^2/s_a^2 |
Sample				
B-1	0.0442	0.0942	0.1384	2.13
B-2	0.0336	0.1185	0.1521	3.53[a]
B-3	0.0614	0.1391	0.2005	2.27
P-1	0.0055	0.0140	0.0195	2.55
P-2	0.0396	0.3713	0.4109	9.38[a]
Fr	0.0223	0.2421	0.2644	10.86[a]
Bol	0.0366	0.1947	0.2313	5.32[a]
All samples	0.0347	0.1649	0.1996	4.75[a]
Homogeneous variance	0.0396[b]	0.2101[c]	0.2301[b]	5.31[a]
	—	0.0541[d]	—	—

[a] Exceeds tabular F-ratio (P = 0.05) indicating variations between means obtained by laboratories differ significantly compared with variations either within laboratory means or variances.
[b] Pooled value for 6 samples excluding lowest variance (P-1).
[c] Pooled value for 5 samples with higher variance: B-2, B-3, P-2, Fr, and Bol.
[d] Pooled value for 2 samples with low variance: B-1 and P-1.

| | | Standard deviation, % fat | | | Rel. dev., % | | | Max. range for detns[a] | |
Sample	Mean, % fat	Repeat-ability, s_a	Be-tween-lab., s_b	Repro-duci-bility, $s_a{}^+{}_b$	CV_a	CV_b	$CV_a{}^+{}_b$	Factor X s_a	Factor X $s_a{}^+{}_b$
B-1	10.77	0.21	0.31	0.37	1.95	2.85	3.45	0.65	1.15
B-2	20.47	0.18	0.34	0.39	0.90	1.68	1.91	0.56	1.21
B-3	25.50	0.25	0.37	0.45	0.97	1.46	1.76	0.78	1.40
P-1	3.49	0.07	0.12	0.14	2.13	3.38	4.00	0.22	0.44
P-2	48.96	0.20	0.61	0.64	0.41	1.24	1.31	0.63	2.02
Fr	27.19	0.15	0.49	0.51	0.55	1.81	1.89	0.47	1.59
Bol	22.62	0.19	0.44	0.48	0.85	1.95	2.13	0.59	1.49
All samples	22.71	0.19	0.41	0.45	0.84	1.81	1.98	0.54	1.42
Homogeneous groups	25.92[b]	0.20[b]	0.46[c]	0.48[b]	0.77	1.59	1.85	0.56	1.51
	7.13[d]	—	0.23[d]	—	—	3.23	—	—	—

[a] 95% confidence level, ±% fat, calculated as noted in footnote a of Table 2.
[b] Sample mean and pooled variance were calculated from respective data of 6 samples, excluding Sample P-1.
[c] Calculated from pooled variance of 5 samples with mean fat content of 28.95%, excluding Samples B-1 and P-1.
[d] Sample mean and pooled variance were calculated from respective data of B-1 and P-1.

it was concluded that: Repeatability of the Foss-Let method on meat and meat products was 0.1% for samples containing 3.5% fat and 0.2% for samples containing >3.5% and ≤49% fat; reproducibility of the method was 0.1% for samples containing 3.5% fat and 0.5% for samples containing >3.5% and ≤49% fat. The maximum ranges for determinations with the method acceptable at a 95% confidence level are shown in the last 2 columns of the table.

Analysis of Variance of Data Sets of All Samples Combined into One Block

Collaborative duplicate determinations were averaged and the day averages were used to calculate 5 sums of squares and 3 mean squares. All determinations from Collaborator 9 were included in an 11-laboratory treatment, and excluded from a 10-laboratory treatment. This was done because the format of this analysis of variance required a complete block of data without a gap that would have resulted by elimination of an outlier. The purpose of the 2 treatments was to obtain averages of the 2 sets of results to express the consensus of method variations. From the mean squares, values of 4 components of precision (Table 5) were calculated. The standard deviation for each component obtained from the 11-laboratory treatment differed very little from that for the same component fro

Table 5. Estimates of precision from analysis of variance between laboratories, samples, and days

Data block	Component of precision			
	Within-lab.	Lab-sample inter-action	Be-tween-lab.	Total of com-ponents
	Variance			
11 labs	0.0421	0.0875	0.0714	0.2010
10 labs	0.0330	0.0971	0.0793	0.2094
	Standard Deviation, % Fat			
11 labs	0.205	0.296	0.267	0.448
10 labs	0.182	0.312	0.282	0.458
Av. of 11- and 10-lab. data	0.19	0.30	0.27	0.45

Table 6. Comparison of fat contents determined collaboratively by Foss-Let and AOAC methods

Sample	Replicate day	Fat, %		Diff. between method means
		Means of detns		
		Foss-Let[a]	AOAC[b]	
B-1	1	10.80	10.82	−0.02
	2	10.75	10.87	−0.12
B-2	1	20.45	20.24	0.21
	2	20.49	20.12	0.37
B-3	1	25.53	25.02	0.51
	2	25.46	25.36	0.10
P-1	1	3.49	3.34	0.15
	2	3.49	3.46	0.03
P-2	1	48.97	48.55	0.42
	2	48.94	48.73	0.21
Fr	1	27.16	27.36	−0.20
	2	27.22	27.51	−0.29
Bol	1	22.64	22.50	0.14
	2	22.59	22.51	0.08
Overall mean	—	22.71	22.60	0.11
Std dev.	—	—	—	0.22
t value	—	—	—	1.87[c]

[a] Means were calculated excluding results for all samples from Collaborator 5 and for Sample P-2 from Collaborator 9.

[b] Means were calculated excluding results for all samples from one collaborator and for Samples Fr and Bol from another (3, Table 2, outliers between days and among laboratories).

[c] This value does not exceed $t = 2.16$ for P = 0.05 and 13 degrees of freedom, indicating that Foss-Let and AOAC methods determine same fat content.

the 10-laboratory treatment. The 4 precisions, directly related to the 3 in Table 4 for all samples, were compared with their counterpart values by using the average of the 11- and 10-laboratory sets of results; they agreed within expected limits. Counterpart values in the 2 tables were compared as follows: Within-laboratory standard deviation agreed with that of its equivalent, repeatability, in Table 4; the sum of the laboratory-sample interaction variance and the simple between-laboratory variance obtained here was used to calculate a standard deviation which was equivalent to and which agreed with the compound between-laboratory standard deviation in Table 4; and the "total of components" standard deviation agreed with that of its equivalent, reproducibility, in Table 4.

Comparison of Precision and Accuracy of the Foss-Let and AOAC Methods

The values of 5 characteristics of precision collaboratively determined for the Foss-Let method showed that fat determination in meat and meat products was at least equivalent to and, except for the simple between-laboratory variation, generally more precise than by the AOAC method, precision characteristics of which were reported in a separate communication (3).

Accuracy of the Foss-Let method was determined to be equivalent to that of the AOAC method from a difference analysis of collaborative means and comparative determinations by the AOAC method. Day means of each sample and between-methods results of the analysis are shown in Table 6. From the overall mean differ-ence, 0.11% fat, the standard deviation of differences between means, 0.22% fat, and 14 differences, a t value indicated (P = 0.05) that the difference between fat determined by the 2 methods was not significant.

Recommendation

We recommend that the Foss-Let method be adopted as official first action as an alternative method for determining crude fat in meat and meat products because of its rapidity of determination (7–10 min) and its accuracy and precision, which were equivalent to those of AOAC 24.005(a) or 24.005(b) in this study.

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REFERENCES

(1) *Official Methods of Analysis* (1975) 12th Ed., AOAC, Washington, DC
(2) Pettinati, J. D., & Swift, C. E. (1975) *JAOAC* 58, 1182–1187
(3) Pettinati, J. D., & Swift, C. E. (1977) *JAOAC* 60, 600–608
(4) Youden, W. J. (1951) *Statistical Methods for Chemists*, John Wiley & Sons, New York, NY
(5) Youden, W. J., & Steiner, E. H. (1975) *Statistical Manual of the AOAC*, AOAC, Washington, DC
(6) *ASTM Annual Book of Standards* (1974) Part 30, American Society for Testing and Materials, Philadelphia, PA, sec. E 180-67
(7) *ASTM Annual Book of Standards* (1975) Part 41, American Society for Testing and Materials, Philadelphia, PA, sec. E 178-75
(8) Snedecor, G. W. (1956) *Statistical Methods*, 5th Ed., The Iowa State University Press, Ames, IA
(9) Sokal, R. R., & Rohlf, F. J. (1969) *Biometry*, W. H. Freeman & Co., San Francisco, CA